General Discussion:

Dementia is responsible for the greatest burden of neurodegenerative diseases, with Alzheimer's Disease (AD) representing approximately 60-70% of dementia cases[1]. To organize optimal individual care for these patients and to predict the disease course, a correct diagnosis is essential. Therefore, a standard clinical work up normally includes a clinical evaluation, neuropsychological tests and imaging of the brain is performed. Clinical diagnosis is established by multidisciplinary team consensus according to the NIA-AA criteria[2]. Since the development of *in vivo* biomarkers, information about the pathophysiological process is becoming more important to diagnose AD[3]. Histopathological hallmarks of AD are deposits of extracellular Amyloid (Aβ) protein and hyper phosphorylated Tau proteins, aggregated in intracellular neurofibrillary tangles[4,5]. Valid diagnostic biomarkers are linked to neuropathology and detect the disease early in its course and distinguish it from other neurodegenerative diseases[6]. This thesis focus on Cerebrospinal fluid (CSF) compounds- and nuclear molecular imaging Positron Emission Tomography (PET) tracer biomarkers to diagnose and stage the different types of dementia.

Summary of main findings

Part 1.

In the first part of the thesis, CSF biomarkers are described and their potential to discriminate AD and different types of dementia. The AD-CSF biomarker profile is characterized by decreased concentration of CSF Aβ and increased concentration of CSF Tau and P-Tau[7]. In **chapter 2,** the perspectives of the established CSF biomarkers and potential new biomarkers in dementia are discussed, with an update of the past and the present. In **chapter 3**, CSF biomarkers $A\beta_{1-42}$, Tau and P-Tau were investigated in a broad population visiting a memory clinic. Baseline CSF was collected from 512 AD patients and 272 patients with other types of dementia, 135 patients with a psychiatric disorder and 275 patients with only subjective memory complaints (SMC). Autopsy was obtained in a subgroup of patients in who neuropathological diagnosis was obtained and the concordance with clinical diagnosis was 85%, while CSF markers reflected neuropathology in 94%. Older patients are more likely to have a CSF AD biomarker profile. We found overlap in CSF biomarker AD profile with other types of dementia, especially in patients with Lewy Body Dementia (DLB, 47%), Corticobasal degeneration (CBD, 38%), and 30% in both Frontotemporal dementia (FTD) and vascular dementia (VaD). Normal CSF biomarker profiles were found in 91% of patients with a psychiatric disorder and in 88% of patients with subjective memory complaints. The conclusion

was, that the AD-CSF biomarkers are useful to diagnose AD, but to differentiate AD from other types of dementia, more specific CSF biomarkers are needed. In **chapter 4**, CSF α-Synuclein was assessed as a biomarker to differentiate DLB and Parkinson Disease (PD), which represent two Parkinson syndromes with α-Synucleinopathy as pathologic hallmark, from AD. We included 35 patients with DLB, 63 AD patients, 18 PD patients and 34 patients with SMC. In our subjects, the CSF α-Synuclein levels could not differentiate between the diagnostic groups ($p = 0.16$). On the other hand, in DLB patients, linear regression analyses of CSF biomarkers showed that lower α-Synuclein was related to lower MMSE and fluency ($p < 0.05$) and thus related to impaired (worse) cognitive performance. Elevation of the AD-CSF biomarker levels P-Tau and Tau, enabled differentiation between AD and DLB patients ($p < 0.05$), although various DLB patients had also a CSF AD profile compared to patients with subjective memory complaints or PD($p < 0.05$). Therefore, we concluded based on this investigation that α-Synuclein CSF biomarker was not useful to differentiate DLB or PD from AD or subjective memory complaints and other biomarkers are needed.

Part 2

In the second part of the thesis, the diagnostic potential of positron emission tomography (PET) biomarkers in dementia was investigated. Molecular imaging techniques, such as amyloid PET ligands, localize and quantify amyloid depositions[8]. [^{11}C]Pittsburgh Compound-B ([^{11}C] (PiB) is an accurate amyloid ligand, although the short half-life of [^{11}C] (20 min), limits its use to centres with a cyclotron and a specialised radiochemistry department. [^{18}F]FDG is a glucose metabolism tracer and as such is a topographic marker for synaptic dysfunction and can thus be applied in a wider field of neurodegeneration than AD[9]. **Chapter 5** does not contain research data but gives an overview of functional imaging and PET biomarkers for FTD, a major cause of young onset dementia. FTD is a heterogeneous syndrome, with different clinical subtypes. Neuropathological characteristics of FTD can be divided in tauopathy (FTD-TAU), ubiquitin pathology (FTD-U or FTD-TDP) or can be associated with Fused in Sarcoma (FUS) protein accumulation (FTD-FUS). Almost half of FTD cases are familial and genetic heterogeneity is reflected by the identification of mutations in causative genes. Diagnostic criteria have modest sensitivity and it is challenging to differentiate FTD from other types of dementia, especially AD. [^{18}F] FDG-PET improves early diagnosis and differentiation of FTD and AD particularly because frontotemporal hypometabolism correlates with the clinical symptoms. Nuclear imaging techniques may also be helpful in the detection of amyloid markers or deficits of the serotonergic, noradrenergic or cholinergic neurotransmitter systems, depending on the degeneration of subcortical nuclei and thereby provide valuable insight in the pathophysiology of FTD. In addition, TAU PET scan is expected to have a role in the diagnosis of specific subtypes of FTD, but Tau tracers are not yet available in clinical practise[10]. In **chapter 6** we identified a DLB-related cerebral glucose metabolic covariance pattern(DLBRP) using a multivariate Scaled Subprofile Model and Principal Component Analysis (SSM PCA) analysis in [^{18}F]FDG PET in 19 healthy controls (HC) and 19 DLB subjects in a Dutch cohort. The

DLBRP expression was subsequently validated in an independent Belgian cohort of 20 HC and 37 DLB subjects. Altered regional FDG PET uptake in DLB is a supportive biomarker for Dementia with Lewy Bodies (DLB), although its diagnostic specificity is not yet clear. SSM PCA derived patterns have shown to be useful in tracking disease progression and differential diagnosis and have advantages over univariate techniques in multiple neurodegenerative diseases, including AD and PD. The DLBRP was characterized by relative hypometabolism in the occipital cortex (including the primary visual cortex), parietal cortex and lateral frontal cortex, covarying with relatively increased metabolism in the brainstem, cerebellum, putamen/pallidum, thalamus, and sensorimotor cortex. FDG standardized uptake value (SUV) in the occipital (visual) cortex was decreased in patients ($p<0.001$) and correlated with the DLBRP in the DLB subjects of the Dutch cohort, a correlation, however, which did not reach statistical significance in the Belgian cohort. These group differences were speculated to relate to the variance in clinical presence of visual hallucinations in the two cohorts and differences in underlying neuropathology. In this, a variable amount of 'pure' neocortical Lewy body pathology was considered[11]. We conclude that ^{18}F-FDG-PET imaging, combined with semi-quantitative multivariate analysis techniques, such as SSM PCA, represents a reliable and valuable biomarker in DLB diagnosis. The clinical implication of this DLBRP to differentiate from other neurodegenerative disease pathology as in AD or PD, needs further investigation. In this respect, future molecular imaging studies targeting α-Synucleinopathy are expected to shed further insights into the pathogenesis of DLB and the relationship between different pathologies. **Chapter 7** describes four out of 29 PIB-positive AD subjects selected from the Dual PET project, i.e. our study with combined ^{18}F-FDG PET and ^{11}C-PiB PET imaging, who exhibited reduced cerebellar FDG uptake associated with reduced uptake in the contralateral cerebral hemisphere. The patients were diagnosed according to the National Institute on Aging - Alzheimer Association (NIA-AA) criteria. PET has additional value on top of the standard diagnostic work-up in AD, especially in early disease or when prior diagnostic confidence is low[12]. A "classic" AD [^{18}F] FDG-PET scan shows hypometabolism in the temporal-parietal association cortex, more variably also in the prefrontal cortex, but with a relative preservation of metabolism in the primary visual and sensorimotor cortex, striatum, and cerebellum[13]. Such prefrontal involvement was particularly the case in the described patients. The cerebellum is often used as a reference region in the analysis of PET brain scans[14], because of the late stage cerebellar involvement in AD in post-mortem studies[15]. Although, [^{18}F] FDG PET AD pattern can also predominantly show focal and lateralized hypometabolism and contralateral cerebellar hypometabolism can be observed, disputing the function as a reference region. This is the first report describing in a small subset of subjects the phenomenon of crossed cerebellar diaschisis (CCD) in AD. CCD is the phenomenon of unilateral cerebellar hypometabolism as a remote effect of supratentorial dysfunction of the brain in the contralateral hemisphere. The mechanism implies the involvement of the cortico-ponto-cerebellar fibers. The pathophysiology is thought to have a functional or reversible basis but can also reflect secondary morphologic change. We found a correlation and interaction between Aβ deposition and

reduced glucose metabolism and a possible role of the bloodflow. We hypothesed that CCD can be explained on a functional basis due to the non-amyloid neurodegenerative pathology in the contralateral (particularly left) hemisphere. In **chapter 8** dual-tracer studies with ^{18}F-FDG and ^{11}C-PIB PET are used to assess respectively metabolism and cerebral amyloid-β deposition in AD. Regional cerebral metabolism and blood flow (rCBF) are closely coupled, both providing an index for neuronal function. The present study compared ^{11}C-PIB-derived rCBF, estimated by the ratio of tracer influx in target regions relative to reference region (R1) and early-stage ^{11}C-PIB uptake (ePIB), to FDG scans. By making this comparison we aimed to assess the use of R1 and ePIB as a surrogate for ^{18}F-FDG. To that end, 15 ^{11}C-PIB positive (+) patients and 15 ^{11}C-PIB negative (-) subjects underwent both FDG and ^{11}C-PIB PET. First, subjects were classified based on visual inspection of the ^{11}C-PIB PET images. Then, discriminative performance (PIB+ versus PIB-) of rCBF methods were compared to normalized regional ^{18}F-FDG uptake. Strong positive correlations were found between analyses, suggesting that the ^{11}C-PIB-derived rCBF provides information that is closely related to what can be seen on ^{18}F-FDG scans. Further studies are needed to validate the use of R1 as an alternative for ^{18}F-FDG studies in clinical applications. **Chapter 9** describes the preliminary results of the 'dual PET' study, i.e. combined ^{11}C-PiB and ^{18}F-FDG PET scans in the clinical diagnosis of AD and other types of dementia. In this ongoing study, 89 subjects were included, with a clinical diagnosis of MCI, probable AD, probable DLB, FTD and healthy controls. For this preliminary analysis, an image-driven approach was chosen to investigate the potential of 'Dual PET' scans in the different patient groups. To that end, a "blinded" expert made a visual judgment of the PET- scans, without knowledge of the clinical symptoms. At first, ^{11}C-PiB PET scans were judged as amyloid positive (n=44) or negative (n=45); next ^{18}F-FDG PET scans were assigned as (i) 'classic' AD pattern, (ii) non- or atypical AD pattern or (iii) a normal ^{18}F-FDG PET pattern. It appeared that the majority of subjects with a clinical diagnosis of AD (18 of 20 subjects) had both a positive [^{11}C]PIB PET scan and a 'classic AD' [^{18}F] FDG PET pattern. The non- or atypical AD [^{18}F] FDG PET patterns associated with [^{11}C] PIB-positive PET scans (18%) concerned subjects that clinically presented as an atypical AD; yielding a 'spectrum' with other neurodegenerative dementias and are assembled in AD-syndromes, as corticobasal syndrome (CBS), primary progressive aphasia (PPA) and posterior cortical atrophy (PCA)[16]. In a minority of subjects (9 %) had normal [^{18}F] FDG PET metabolism and a positive [^{11}C] PIB PET scan, suggesting an early sign of disease[17]. In most of the [^{11}C] PIB- PET negative scans, subjects were clinically diagnosed as FTD. In 35 % of [^{11}C] PIB- PET negative subjects, the [^{18}F] FDG PET pattern was classified as "classic AD". The clinical diagnosis of these patients comprised DLB, FTD, other dementia and obstructive sleep apnea syndrome (OSAS). Therefore, [^{18}F] FDG PET scan is not specific, but has an added negative predictive value[18] to exclude (other types of) dementia. The preliminary conclusion was that this adjustments after 'Dual PET' analysis match the clinical diagnosis. Further analysis of clinical and neuropsychological subtypes in this cohort will follow.

Biomarkers to diagnose dementia:

Biomarkers ideally provide an early stage diagnosis of dementia, enabling the differentiation between distinct types of neurodegenerative diseases, while they may additionally serve as a marker of disease progression. A correct diagnosis is an indispensable starting point for dementia research and future therapies. On the other hand, a cure for dementia is still missing. Therefore, to consider the application of dementia biomarkers in general practice is an individual choice, and depends on the specific needs of the patient and his/her family. In practice, it concerns 'shared decision making' which implies that patients have the right to either be informed, or remain unknown about their diagnosis. Also cost effectiveness is an important issue to consider. If future treatment trials start to be effective, the role of dementia biomarkers will enhance.

CSF biomarkers to diagnose AD:

CSF biomarkers for AD (low levels of Aβ with high levels of t-tau and p-tau) have high sensitivity and specificity compared with cognitively normal individuals[19] and are embedded in guidelines and clinical criteria of AD[20]. CSF analysis is relatively inexpensive and can be performed in a broad clinical setting and is therefore a preferred tool for work up in AD. CSF Aβ42 has a robust correlation with pathology and is an early marker of AD[21]. CSF T-Tau and P-Tau are also increased in AD, but not in several other tauopathies[22]. A high CSF Tau concentration correlates with rapid disease progression[23]. Although, in meta-analysis, CSF biomarkers have not yet reached the required validation level to be routinely applied in the clinic[24]. One of the reasons is that there is substantial overlap in abnormal CSF AD markers in several other dementia syndromes[25]. In real-life clinical setting, 'pure' etiologies among dementia syndromes are relatively rare and AD neuropathology is found in several other neurodegenerative diseases[26]. CSF biomarkers of AD can also be abnormal in cognitively unimpaired individuals[27] and in prodromal stage of disease[28]. As described in chapter 3, the CSF AD biomarker profile raises with older age[29,30] and is also found in DLB (47%), CBD (38%), FTD (30%) and VaD (30%). Meanwhile, an evolution has taken place in neuropathological criteria of dementia syndromes to clinicobiological entities and *in vivo* biomarkers, reflecting the disease-specific pathophysiological processes[26]. The NIA-AA working group recently defined AD biomarkers for diagnostic research criteria and therapeutic trials, grouped into amyloid-β deposition, tau pathology and neurodegeneration in the 'A/T/N classification'[3].

According to amyloid biomarkers: The sensitivity and specificity of CSF Aβ42 is overall lower than amyloid PET[24]. The concordance of amyloid CSF and amyloid PET markers are 60%[31] to 80%[32], although a more specific ratio of CSF Aβ42/Aβ40 can partly correct for disconcordance[33,34]. In chapter 8 we assembled 14 AD patients which underwent both PIB PET and CSF examination and found a concordance of 54% in [^{11}C]PIB- positive subjects and 93% in [^{11}C]PIB-PET negative subjects. If CSF results are doubtful,

conflicting or 'borderline'[35], there is an additional value of amyloid PET scan of 35%[36]. In case that lumbar puncture is not applicable, as it may be refused, not be successful or not possible because of risk factors, PET biomarkers may be appropriate.

[^{18}F]FDG PET biomarkers to diagnose different types of dementia:

[^{18}F]FDG PET brain scan is commonly used in general practice as a topographic marker of synaptic dysfunction and is related to neurodegenerative diseases and dementias[9]. Specific metabolic patterns of disease provide a deeper insight into the interplay between topography of neurodegeneration and clinical presentation. How [^{18}F]FDG PET scan can lead to new insights in mechanisms underlying the topography of dysfunction is shown by the phenomenon of crossed cerebellar diaschisis (CCD) in AD as we described in Chapter 7. Pathophysiological mechanism of reduced glucose metabolism and [^{18}F]FDG PET hypometabolism correlates with the bloodflow, and may be reversible or reflect morphologic change. This strong positive correlation in regional cerebral bloodflow and decreased metabolism is also demonstrated in the dual (FDG and PiB PET)-tracer study described in Chapter 6. As shown in the preliminary results of the 'Dual PET 'study (Chapter 8): [^{18}F]FDG PET scan can be assessed in the work up, if more than one class of dementia is possible to diagnose or when an atypical AD variant is suspected. [^{18}F]FDG PET is the main tool to ascertain atypical AD variants such as PPA or PCA[36] and improves the differentiation of FTD[37] and DLB[38]. Combined with optimized semi-quantitative multivariate approaches such as SSM PCA, [^{18}F]FDG PET can be an even more effective tool to support diagnosis in a quantitative fashion. [^{18}F]FDG PET scan has a high negative predictive value and a normal scan rules out a neurodegenerative disease. For example, in depressed patients an underlying neurodegenerative disease may be suspected. In identifying prodromal AD in MCI patients, the [^{18}F]FDG PET scan has a similar accuracy[39] and is therefore an alternative to CSF biomarkers, especially in older age- with decreased CSF specificity. [^{18}F]FDG PET scan may function as a non-specific progression marker of neurodegenerative disease[40] and can therefore also be used as a surrogate outcome measure in clinical trials. A limitation of [^{18}F]FDG PET is the lack of specificity in a heterogeneous disease spectrum and in mixed dementias and specific neuropathological markers may then be needed.

The role of amyloid PET in clinical practice:

Amyloid PET is a specific biomarker with a sensitivity around 95%[41], and is therefore currently the best tool to rule out AD diagnosis[24]. The [^{11}C]-PiB PET ligand is capable to trace insoluble amyloid in the brain[42] but can also be positive in other diseases, such as cerebral amyloid angiopathy in patients without dementia[43]. In clinical practice, amyloid PET is employed in case of an unclear diagnosis to (i) rule out or confirm diagnosis of AD; (ii) gain support for an atypical presentation with AD CSF profile, or (iii) further

evaluate the clinical suspicion of AD in case of negative or unclear CSF results. Also, early-stage ^{11}C-PIB uptake may function as a surrogate for ^{18}F-FDG PET, providing an index for neuronal function[44]. The use of amyloid PET is still very limited in Europe and the United States, because the costs are not reimbursed by the majority of health providers. The Alzheimer Biomarker in Daily Practice study is a clinical study to assess the diagnostic value of amyloid PET scan in clinical practise[45]. The 'Dual PET' study extends on this by investigating how the different PET modalities, increase the clinical accuracy. Future analysis of the 'Dual PET' study data, including multivariate analysis of multiple PET tracers, will focus on the correlation between clinical symptoms, amyloid accumulation and neurodegeneration as revealed by regionally impaired ^{18}F-FDG uptake. In this, particularly (lateralized) dementia cases may provide further insight in underlying disease mechanism.

Future biomarkers for dementia:

Several future promising biomarkers for the differential diagnosis of dementia have become available, of which particularly the development of Tau tracers for PET (such as THK5317, THK5351, AV-1451 and PBB3) raise high expectations. To assess their usefulness as an early biomarker of the underlying pathology, there is still work to be done in order to characterize the binding properties for the variety and complexity of the various types of tau deposits in different diseases[46,47]. New candidate biomarkers for AD or other dementias are still under investigation, but sensitivity and specificity analysis are not yet applicable for clinical practice[6]. Specific α-Synucleine biomarkers to diagnose Parkinsonian syndromes and DLB are still an unmet need. Novel ultrasensitive immunoassays and mass spectrometry methods are being investigated for future clinical practice[48]. Recent technical development of serologic amyloid biomarkers may have potential in the enrichment of AD therapeutic trials. Serologic biomarker 'Neurofilament light' is a general marker of neurodegeneration and could be used as a marker of progression[49]. Also multivariate analysis of large sets of serum proteins can be expected to achieve a high level of accuracy to predict AD diagnosis[50,51].

From biomarkers to clinical and therapeutic trials:

The majority of biomarkers used in therapeutic trials are based on the 'amyloid cascade' hypothesis, i.e. the imbalance between production and clearance of the Aβ protein[52], which leads to toxic deposits of extracellular amyloid plaques[53, 54] and aggregates of intracellular neurofibrillary tangles[4, 55], causing widespread neurodegeneration[56]. In our center we are currently involved in a phase -1 clinical trial, based on RIPK1 as the therapeutic target[57] to reduce amyloid burden, inflammatory cytokines and memory deficits[57]. Another recently initiated phase 1 trial is a promising therapeutic approach for primary and secondary tauopathies[58] employing an antisense oligonucleotide,

developed to reduce Tau expression mRNA. Future drug design methods will potentially focus on multi-target pharmacological treatment of AD[59]. However, also other concepts concerning complex and multifactorial disease mechanism in dementia may generate hypotheses that lead to pioneering research and therapeutic trials. In this respect, the notion that the blood-brain barrier (BBB) is a endothelial protecting membrane to neurotoxic components and pathogens[60] ,with the consequence that BBB dysfunction leads to neurodegeneration, is one of such hypotheses. Targeting the BBB dysfunction forms the basis for developing new dementia therapies[61]. In our research group, molecular PET tracers, as (R)-[11C]verapamil, are developed to quantify the efflux function of P-glycoprotein, an ABC transporter of the BBB and new PET probes are focused on improved characteristics[62]. Another hypothesis to target disease modifying strategies of neurodegenerative diseases, is the focus on noradrenergic loss of Locus Coeruleus (LC) projection neurons, mediating attention, memory and arousal[63]. In our center we apply [^{11}C] Methylreboxetine (MRB)[64], a selective PET imaging biomarker, to measure noradrenaline transporter (NET) availability in LC projection neurons in neurodegenerative disorders (AD, DLB and PD). Another important hypothesis of neurodegenerative mechanism of diseases is based on the cell's protein degradation of the ubiquitin proteasome system (UPS) causing misfolded proteins, and future treatment targets are directed to this proteasome[65].

To conclude, biomarkers for dementia are important in clinical practice as well as research to diagnose and stage AD and other types of dementia. Combining high standard clinical practice and clinical research will hopefully lead to the future therapies for dementia.

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